NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 430

TOXICOLOGY AND CARCINOGENESIS STUDIES OF C.I. DIRECT BLUE 218

(CAS NO. 28407-37-6)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

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(FEED STUDIES)

A desalted commercial dye containing approximately 60% copper complex of 3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt, 1% sodium chloride, 9% water, and 30% unknown

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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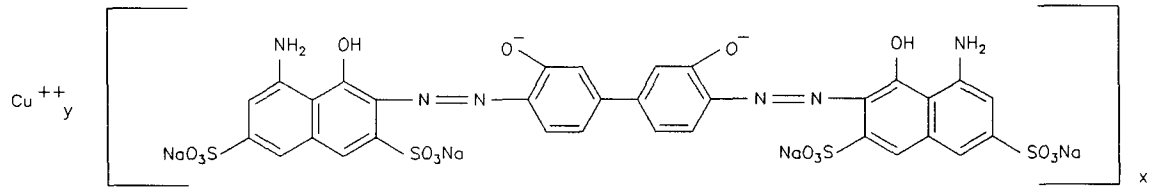
CONTENTS

ABSTRACT

C.I. DIRECT BLUE 218

CAS No. 28407-37-6



Major Component of C.I. Direct Blue 218

A copper complex of 3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis [5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt

Chemical Formula: $(C_{32}H_{18}N_6O_{16}S_4Na_4)_xCu_y$ Molecular Weight: Approx. 1,090 (assumes 2 copper ions per molecule)

Synonyms: cuprate(4-), [μ-[[3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonato]](8-)]]di-, tetrasodium; copper, [tetrahydrogen-3,3′-[(3,3′-dihydroxy-4,4′-biphenylylene) bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonato](4-)]di-, tetrasodium salt; 1-naphthol-3,6-disulfonic acid, 2,2′-(3,3′-dihydroxy-4,4′-biphenylylenebisazo)bis[8-amino-, dicopper deriv., tetrasodium salt

Trade Names: Amanil Supra Blue 9GL; Carta Blue VP; Fastusol Blue 9GLP; Pontamine Bond Blue B; Solantine Blue 10GL; Pontamine Fast Blue 7GLN;

C.I. Direct Blue 218 is a copper chelated dye used for cellulose, acetate, nylon, silk, wool, tissue, papers, and textile goods with a urea-formaldehyde finish. C.I. Direct Blue 218 is one of five chemicals/dyes that are part of the National Toxicology Program's Benzidine Dye Initiative, established to determine the toxicity and carcinogenicity of representative benzidine congeners, congener-derived dyes, and benzidine-derived dyes. Industrial grade C.I. Direct Blue 218 was selected for study because of its widespread use. Because of the high salt content, the dye was desalted prior to use. Toxicology and carcinogenesis studies were conducted by administering C.I. Direct Blue 218 in feed to groups of male and female F344/N rats and B6C3F$_1$ mice for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, and *Drosophila melanogaster*.

14-Day Study in Rats

Groups of five male and five female F344/N rats were fed diets containing 0, 1,000, 3,000, 7,000, 15,000, or 30,000 ppm C.I. Direct Blue 218. All rats survived until the end of the study. Rats receiving 30,000 ppm lost weight, and the mean body weight gain of males receiving 15,000 ppm was significantly lower than that of the controls. Feed consumption by rats receiving 30,000 ppm was lower than that by the controls. Decreased organ weights at the 30,000 ppm level were related to the decreased body weights at this exposure level.

14-Day Study in Mice

Groups of five male and five female mice were fed diets containing 0, 1,000, 3,000, 7,000, 15,000, or

30,000 ppm C.I. Direct Blue 218. All mice survived until the end of the study. The final mean body weight of males receiving 30,000 ppm was 25% lower than that of controls and that of 30,000 ppm females was 20% lower than that of controls. Feed consumption by exposed and control groups was similar except for the 15,000 and 30,000 ppm groups. Feed spillage, due to reduced palatability, precluded the accurate determination of feed consumption by these two groups. Male and female mice receiving 30,000 ppm appeared hyperactive and emaciated during the last week of the study. Decreased organ weights were noted at 30,000 ppm and were attributed to the decreased mean body weights at this exposure level.

13-Week Study in Rats

Groups of 10 male and 10 female rats were fed diets containing 0, 3,000, 10,000, or 20,000 ppm C.I. Direct Blue 218. All male and female rats survived until the end of the study. Rats exposed to 3,000, 10,000, or 20,000 ppm C.I. Direct Blue 218 received approximate daily doses of 200, 600 or 1,300 mg dye/kg body weight (males) and 200, 800, or 1,400 mg/kg (females). The final mean body weight of male rats receiving 20,000 ppm was 24% lower than that of the controls and the final mean body weight of female rats receiving 20,000 ppm was 15% lower than that of the controls. Feed consumption by exposed and control groups was similar except in the 20,000 ppm groups where feed spillage was noted. Absolute and relative kidney weights of rats receiving 10,000 or 20,000 ppm were significantly greater than those of controls. Significantly decreased organ weights were noted, particularly in the 20,000 ppm groups, and were attributed to the lower mean body weights at this exposure level.

The hematocrit, hemoglobin, mean erythrocyte volume, and mean erythrocyte hemoglobin values in male and female rats receiving 10,000 and 20,000 ppm were significantly lower than those of controls. Serum levels of alanine aminotransferase and sorbitol dehydrogenase in male and female rats receiving 20,000 ppm were significantly higher than those of controls, which is consistent with hepatocellular injury. Male rats receiving 10,000 ppm and male and female rats receiving 20,000 ppm had hepatic lesions consisting of intracytoplasmic pigment in periportal Kupffer cells, minimal to mild individual hepatocyte necrosis, increased numbers of binucleated and multinucleated hepatocytes, and minimal bile duct hyperplasia. Male and female rats receiving 20,000 ppm had yellow-green pigment within the cytoplasm of proximal convoluted tubules of the kidney. Microconcretions of mineral were observed along the corticomedullary junction of the kidney in most female rats, but the numbers of microconcretions in kidney sections were increased in females that received 20,000 ppm.

13-Week Study in Mice

Groups of 10 male and 10 female B6C3F$_1$ mice were fed diets containing 0, 3,000, 10,000, or 20,000 ppm C.I. Direct Blue 218. There were no deaths attributed to C.I. Direct Blue 218. Mice exposed to 3,000, 10,000, or 20,000 ppm C.I. Direct Blue 218 received approximate daily doses of 400, 1,500, or 3,600 mg dye/kg body weight (males) and 400, 1,800, or 4,000 mg/kg (females). The final mean body weight of males that received 20,000 ppm was 24% lower than that of the controls, and the final mean body weight of females that received 20,000 ppm was 14% lower than that of controls. Feed consumption by exposed mice was similar to that by controls except in the 20,000 ppm groups where feed spillage was noted. Significant differences in organ weights were noted at 20,000 ppm which were attributed primarily to the lower mean body weights in these exposure groups.

The hematocrit, hemoglobin, mean erythrocyte volume, and mean erythrocyte hemoglobin values were significantly lower in males and females receiving 10,000 and 20,000 ppm. Serum levels of alanine aminotransferase and sorbitol dehydrogenase in male and female mice receiving 10,000 and 20,000 ppm were significantly higher than those of controls, indicating hepatic injury. Male and female mice receiving 20,000 ppm had hepatic lesions consisting of centrilobular hepatocyte hypertrophy and karyomegaly, multifocal individual hepatocyte necrosis, oval cell proliferation, and periportal Kupffer cells with intracytoplasmic pigment. Males and females receiving 20,000 ppm also had increased numbers of pigmented macrophages within the red pulp of the spleen.

2-Year Study in Rats

The doses selected for the 2-year study of C.I. Direct Blue 218 were based on the lower final mean body weights and the occurrence of hepatic lesions in the

20,000 ppm groups in the 13-week study. Groups of 60 male and 60 female rats were fed diets containing 0, 1,000, 3,000, or 10,000 ppm C.I. Direct Blue 218 for 103 weeks. Nine or 10 rats from each group were evaluated after 15 months.

Survival, Body Weights, Feed and Compound Consumption, and Clinical Findings

Survival of female rats receiving 10,000 ppm was slightly, but not significantly, lower than that of the controls. Mean body weights of male and female rats in the 10,000 ppm groups were approximately 5% to 14% lower than those of the controls after week 15, and the final mean body weights of male and female rats at this level were 11% and 9% lower than those of the controls, respectively. Feed consumption by exposed male and female rats was similar to that by the controls and was estimated to deliver daily doses of 40, 120, and 440 mg dye/kg body weight to males and 50, 140, and 470 mg/kg to females. No chemical-related clinical signs of toxicity were noted.

Hematology and Clinical Chemistry

The hematocrit, hemoglobin, mean erythrocyte volume, and mean erythrocyte hemoglobin values in 10,000 ppm female rats were significantly lower than those of controls, while in males only the mean erythrocyte hemoglobin value was significantly lower. Serum levels of alanine aminotransferase and sorbitol dehydrogenase in male and female rats receiving 10,000 ppm were significantly higher than those of the controls at the 15-month interim evaluation.

Pathology Findings

Squamous cell papillomas of the oral mucosa (pharynx) occurred in five males receiving 10,000 ppm but not in the lower exposure groups or in controls. A squamous cell carcinoma occurred in one 10,000 ppm male and a benign basosquamous tumor was observed in another. The incidence of oral mucosal neoplasms in the 10,000 ppm males was significantly greater than that in controls and exceeded the range observed in untreated historical controls (10/1,253, 0.8%; range 0%-4%). These neoplasms were considered chemical related.

Administration of C.I. Direct Blue 218 to rats produced significantly increased incidences of forestomach basal cell hyperplasia in males receiving 3,000 or 10,000 ppm (0 ppm, 0/50; 1,000 ppm, 2/50; 3,000 ppm, 10/50; 10,000 ppm, 19/50) and in females receiving 10,000 ppm (1/50, 1/49, 5/50, 11/49). Further, there were marginal increased incidences of focal squamous hyperplasia in the 3,000 and 10,000 ppm males (1/50, 1/50, 6/50, 4/50). Squamous cell papillomas of the forestomach were seen in two 3,000 ppm males and in one 10,000 ppm male; no papillomas were observed in the controls. A squamous cell carcinoma was also seen in one 3,000 ppm male. Because of the uncommon occurrence of forestomach neoplasms in untreated control male rats (4/1,253, 0.3%; range 0%-2%) and the slight increase in the incidence of focal hyperplasia, these neoplasms may have been chemical related.

The incidence of uterine endometrial stromal polyps in each exposed group of female rats was significantly greater than that of the controls (1/50, 12/50, 10/50, 10/50). Because the incidences in the exposed groups did not increase in a dose-related manner and the incidence in the controls was unusually low (historical incidence: 205/1,251, 16.4%; range 2%-30%), the higher incidence of stromal polyps in the exposed groups was not considered chemical related.

2-YEAR STUDY IN MICE

The dose selection for the 2-year study was based on the lower final mean body weights and the liver lesions observed at the 20,000 ppm level in the 13-week study. Groups of 60 male and 60 female mice were fed diets containing 0, 1,000, 3,000, or 10,000 ppm C.I. Direct Blue 218 for 103 weeks. Nine or 10 mice from each exposure group were evaluated after 15 months.

Survival, Body Weights, Feed and Compound Consumption, and Clinical Findings

Survival of exposed male and female mice was similar to that of the controls. Mean body weights of male and female mice receiving 10,000 ppm were 10% to 29% lower than those of the controls during most of the study, and the final mean body weights in these groups were 19% lower than that of the controls for males and 27% lower than that of the controls for females. Feed consumption by exposed mice was similar to that by controls and the diets were estimated to deliver daily doses of approximately 120, 360, and 1,520 mg of dye/kg body weight to males and 140, 470, and 2,050 mg/kg to females. No chemical-related clinical signs of toxicity were noted.

Hematology and Clinical Chemistry

Hematocrit, hemoglobin, and mean erythrocyte volume values in males and females receiving 10,000 ppm were significantly lower than those of the controls. Serum levels of alanine aminotransferase and/or sorbitol dehydrogenase values in male and female mice that received 10,000 ppm were significantly higher than those of controls, which is consistent with hepatocellular damage.

Pathology Findings

The administration of C.I. Direct Blue 218 to mice produced significantly increased incidences of hepatocellular adenoma (0 ppm, 16/50; 1,000 ppm, 19/50; 3,000 ppm, 17/50; 10,000 ppm, 40/50) and hepatocellular carcinoma (7/50, 3/50, 8/50, 17/50) in males receiving 10,000 ppm, and a significantly increased incidence of hepatocellular adenoma in females receiving 3,000 or 10,000 ppm (7/49, 12/50, 17/49, 41/49). In females that received 10,000 ppm, the incidence of hepatocellular carcinoma was marginally increased. Consistent with these findings, the incidence of hepatocellular foci of cytologic alteration, a preneoplastic lesion, was also increased in males and females in the 10,000 ppm groups. The increased incidences of hepatocellular foci, adenomas, and carcinomas were considered chemical related.

Uncommon renal tubule neoplasms also occurred at low incidences in male mice receiving C.I. Direct Blue 218, but not in controls. Renal tubule adenomas were seen in two males receiving 1,000 ppm, one male receiving 3,000 ppm, and one male receiving 10,000 ppm. A renal tubule carcinoma was also seen in one male that received 1,000 ppm. Because renal tubule neoplasms are uncommon in male mice (4/1,366, 0.3%; range 0%-2%), these neoplasms may have been chemical related.

Carcinomas of the small intestine occurred in four male mice receiving 10,000 ppm. One was observed at the 15-month interim evaluation, while the other three were observed in mice at the end of the study. One control male mouse also had a carcinoma of the small intestine. Because of the uncommon occurrence of small intestine neoplasms in untreated male mice (12/1,374, 0.9%; range 0%-4%), the slightly higher incidence of these neoplasms in males receiving 10,000 ppm may have been chemical related. Carcinomas of the small intestine also occurred in one 3,000 ppm and one 10,000 ppm female, but the low incidences precluded drawing an association with chemical administration.

GENETIC TOXICOLOGY

C.I. Direct Blue 218 was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 tested with and without exogenous metabolic activation (S9). It was also tested in a modified *Salmonella* test protocol which employed reductive metabolism supplied by flavin mononucleotide or rat cecal bacteria, followed by oxidative metabolism; results of this test using strain TA1538 were also negative. C.I. Direct Blue 218 induced a small but significant increase in sister chromatid exchanges in Chinese hamster ovary cells at the highest dose tested without S9. No increase in chromosomal aberrations were observed in Chinese hamster ovary cells with or without S9. C.I. Direct Blue 218 did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster.*

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *some evidence of carcinogenic activity** of C.I. Direct Blue 218 in male F344/N rats based on the occurrence of pharyngeal neoplasms. Squamous cell neoplasms of the forestomach may have been chemical related. There was *no evidence of carcinogenic activity* of C.I. Direct Blue 218 in female F344/N rats given 1,000, 3,000, or 10,000 ppm. There was *clear evidence of carcinogenic activity* of C.I. Direct Blue 218 in male and female B6C3F_1 mice based on increased incidences of hepatocellular adenomas and carcinomas. The occurrence of a few neoplasms of the kidney and small intestine in male mice may have been related to C.I. Direct Blue 218 treatment.

The administration of C.I. Direct Blue 218 produced an increased incidence of forestomach basal cell hyperplasia in rats and hepatocellular foci of cytologic alteration in mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of C.I. Direct Blue 218

Variable	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 1,000, 3,000, or 10,000 ppm in feed (0, 40, 120, or 440 mg/kg)	0, 1,000, 3,000, or 10,000 ppm in feed (0, 50, 140, or 470 mg/kg)	0, 1,000, 3,000, or 10,000 ppm in feed (0, 120, 360, or 1,520 mg/kg)	0, 1,000, 3,000, or 10,000 ppm in feed (0, 140, 470, or 2,050 mg/kg)
Body weights	High-dose group lower than controls	High-dose group lower than controls	High-dose group lower than controls	High-dose group lower than controls
2-Year survival rates	30/50, 25/50, 29/50, 24/51	35/51, 29/51, 31/50, 25/50	44/50, 46/50, 42/50, 45/50	37/49, 40/50, 46/49, 38/49
Nonneoplastic effects	Forestomach: basal cell hyperplasia (0/50, 2/50, 10/50, 19/50)	Forestomach: basal cell hyperplasia (1/50, 1/49, 5/50, 11/49)	Liver: eosinophilic foci (13/50, 12/50, 10/50, 28/50); all foci (combined) 22/50, 22/50, 16/50, 32/50	Liver: eosinophilic foci (11/49, 7/50, 11/49, 21/49); all foci (combined) 13/49, 11/50, 17/49, 29/49
Neoplastic effects	Pharynx: squamous cell papilloma (0/50, 0/50, 0/50, 5/50); squamous cell carcinoma (0/50, 0/50, 0/50, 1/50); basosquamous tumor benign (0/50, 0/50, 0/50, 1/50)	None	Liver: hepatocellular adenoma (16/50, 19/50, 17/50, 40/50); hepatocellular carcinoma (7/50, 3/50, 8/50, 17/50)	Liver: hepatocellular adenoma (7/49, 12/50, 17/49, 41/49); hepatocellular carcinoma (5/49, 5/50, 6/49, 12/49)
Uncertain findings	Forestomach: squamous cell papilloma (0/50, 0/50, 2/50, 1/50); squamous cell carcinoma (0/50, 0/50, 1/50, 0/50)	None	Kidney (renal tubule): adenoma (0/50, 2/50, 1/50, 1/50); carcinoma (0/50, 1/50, 0/50, 0/50); adenoma or carcinoma (0/50, 3/50, 1/50, 1/50) Small intestine: carcinoma (1/50, 0/50, 0/50, 3/50)	None
Level of evidence of carcinogenic activity	Some evidence	No evidence	Clear evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutation:	Negative with and without S9 in strains TA98, TA100, TA1535, and TA1537			
Salmonella typhimurium with reductive metabolism:	Negative in strain TA1538			
Sister chromatid exchanges Chinese hamster ovary cells *in vitro*:	Weakly positive without S9; negative with S9			
Chromosomal aberrations Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Sex-linked recessive lethal mutations *Drosophila melanogaster*:	Negative when administered in feed or by injection			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on C.I. Direct Blue 218 on December 1, 1992, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On December 1, 1992, the draft Technical Report on the toxicology and carcinogenesis studies of C.I. Direct Blue 218 received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of C.I. Direct Blue 218 by discussing the use and rationale for study (as part of the NTP Benzidine Dye Initiative), describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions were *some evidence of carcinogenic activity* for male F344/N rats, *no evidence of carcinogenic activity* for female F344/N rats, and *clear evidence of carcinogenic activity* for male and female B6C3F$_1$ mice.

Dr. Davidson, a principal reviewer, agreed with the proposed conclusions. She said the background information on the metabolism, toxicity, and carcinogenicity of benzidine and benzidine-congener based dyes was detailed and appeared representative of the available literature.

Mr. Beliczky, the second principal reviewer, agreed with the proposed conclusions. He thought a tabular reference chart comparing results for various benzidine dye derivatives should be included (Table 21).

Dr. Ward commented on the incidence of three kidney neoplasms in male mice at the lowest dose, wondering why this finding did not fall under *clear evidence* as these are rare neoplasms and there was a corresponding lack of renal toxicity. Dr. Dunnick responded that the lack of a dose response and the lack of statistical significance compared with the controls led to the conclusion that these were only uncertain findings. Dr. Davis questioned the rationale for the oral route of administration being selected to mimic exposure in the home and workplace, suggesting that dermal exposure was more likely. Dr. Ryan noted the teratogenic effects reported for the benzidine-based dyes and suggested reproductive and developmental toxicology studies would be appropriate. Dr. Dunnick reported that at the time the benzidine-based dye studies were initiated, potential for carcinogenesis was a primary concern, but agreed that reproductive effects should be, and are receiving, increasing priority.

Dr. Davidson moved that the Technical Report on C.I. Direct Blue 218 be accepted with the revisions discussed and with the conclusions as written for male rats, *some evidence of carcinogenic activity,* for female rats, *no evidence of carcinogenic activity,* and for male and female mice, *clear evidence of carcinogenic activity.* Mr. Beliczky seconded the motion, which was accepted unanimously with ten votes.